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Letter to the Editor

Full Resistance and Decreased Susceptibility to Carbapenems in IMP-13-Producing *Pseudomonas aeruginosa* Isolates from an Outbreak▽

Of 23 ceftazidime-resistant *Pseudomonas aeruginosa* isolates with simultaneous decreased susceptibility to carbapenems recovered from inpatients at Hospital Eva Peron, Provincia de Buenos Aires, Argentina, from December 2004 to December of 2005, 18 were positive by a double-disk phenotypic screening of metallo-β-lactamases (MBLs) using EDTA (1 μmol) (6). Decreased susceptibility was defined as to refer to isolates with MICs of 2 to 8 μg/ml or inhibition zones of 16 to 21 mm, categorized as susceptible or intermediate according CLSI breakpoints, but which clearly differed from fully susceptible isolates.

All of these isolates could be categorized as susceptible to piperacillin, piperacillin-tazobactam, and colistin. Thirteen of 18 isolates were susceptible to amikacin, while 5/18 were intermediate. They were all resistant to ceftazidime, cefepime, gentamicin, and ciprofloxacin. From them, 14 displayed decreased susceptibility to imipenem (IPM) and meropenem (MEM) (inhibition zone range, 16 to 21 mm), while 4 (consecutive) isolates were considered fully resistant to both (Table 1). Independently of their categorization, pulsed-field gel electrophoresis (PFGE) profiles using 20 U/plug SpeI nuclease (NEB) were identical after standard resolution (4).

These 18 isolates were positive when screened for the class 1 integrase gene and rendered a unique fragment corresponding to the variable region of class 1 integrons, in which *imp-13* was the first cassette, followed downstream by an aminoglycoside-modifying enzyme-coding gene, *aacA4* (AM931299), 99% identical to that already deposited for Tn*5051* (5, 9).

Because a difference in final carbapenem resistance could be due to different mechanisms, including differential β-lactamase content and expression, crude extracts were examined after analytical isoelectrofocusing, detecting enzymatic activity with

TABLE 1. Epidemiological data and antimicrobial resistance profile[a]

Isolate	Age (yr)/ sex	Admission date (mo/day/yr)	Underlying condition[b]	Ward[c]	Diagnosis[d]	Culture source[e]	Empirical therapy[f]	Date of isolation (mo/day/yr)	Antibiotic susceptibility interpretation[g]				Therapeutic treatment[f]	Outcome
									IPM		MEM			
									DD (mm)	MIC (μg/ml)	DD (mm)	MIC (μg/ml)		
1	65/male	11/26/04	—	ICU	ABS	AF	SAM, TZP, CIP, IPM	12/14/04	20 (S)	4 (S)	18 (S)	4 (S)	CST	Died
2	69/female	12/15//04	AH, SK	ICU	EVS	BL/CAT	VAN, SXT, AMK	12/31/04	21 (S)	2 (S)	17 (S)	4 (S)	CST	Died
3	60/male	12/30/04	AMI, AH	ICU	Sepsis	BL	SAM, GEN	1/11/05	20 (S)	2 (S)	16 (S)	4 (S)	CIP, SAM, GEN	Died
4	57/male	3/25/05	SCZ, SK, COL	ICU	VAP	BAL	CRO, CIP, TZP, IPM, SXT	4/7/05	21 (S)	2 (S)	21 (S)	4 (S)	SXT, IPM	Died
6	56/female	2/28/05	DM, CRF	GW	SSTI, OS	BO	Unknown	5/16/05	21 (S)	2 (S)	17 (S)	4 (S)	Unknown	Favorable
5	31/male	5/11/05	—	ICU	VAP	BAL	CAZ, VAN	5/18/05	20 (S)	2 (S)	16 (S)	2 (S)	PIP, AMK	Died
7	86/female	5/10/05	DM	ICU	CAI	CAT	CRO, CAZ, AMK, VAN	5/26/05	21 (S)	2 (S)	17 (S)	4 (S)	IPM	Died
8	22/male	5/28/05	SK	ICU	VAP	BAL		6/3/05	21 (S)	2 (S)	21 (S)	4 (S)	CIP, AMK	Favorable
9	49/male	5/27/05	COPD CRF	ICU	VAP	BAL	SAM, CIP	7/9/05	18 (S)	2 (S)	19 (S)	2 (S)	IPM, CST	Died
10	42/male	7/05/05	—	ICU	VAP	BAL	CRO, CLI	7/9/05	21 (S)	2 (S)	20 (S)	4 (S)	IPM, CST	Died
11	67/male	7/08/05	AH	ICU	VAP	BAL	CIP, AMK	7/15/05	21 (S)	2 (S)	19 (S)	4 (S)	IPM	Favorable
12	79/male	7/15/05	DV, HF	ICU	VAP	BAL	SAM, CIP	7/23/05	18 (S)	4 (S)	17 (S)	8 (I)	IPM, CST	Died
13	**27/female**	**8/14/05**	**—**	**ICU**	**VAP**	**BAL**	**TZP, VAN, CAZ, IPM, SAM**	**12/10/05**	**6 (R)**	**32 (R)**	**6 (R)**	**128 (R)**	**TZP**	**Favorable**
14	**31/male**	**12/5/05**	**—**	**GW/S**	**SSTI**	**ST**	**IPM, VAN**	**12/11/05**	**6 (R)**	**32 (R)**	**6 (R)**	**128 (R)**	**IPM, VAN, CST**	**Died**
15	**33/male**	**12/19/05**	**—**	**GW**	**SSTI**	**ST**	**CFZ, GEN, ERY**	**12/21/05**	**7 (R)**	**32 (R)**	**6 (R)**	**128 (R)**	**IPM, AMK**	**Favorable**
16	**36/female**	**12/19/05**	**DM, CRF**	**GW**	**CAI**	**BO/CAT**	**IPM, VAN**	**12/30/05**	**6 (R)**	**32 (R)**	**6 (R)**	**128 (R)**	**AMK, CST**	**Died**
17	33/male	12/19/05	—	GW/S	SSTI	ST	CFZ, GEN	1/2/06	20 (S)	2 (S)	20 (S)	2 (S)	IPM, AMK	Favorable
18	56/male	12/9/05	VL	ICU	PSP	AF	IPM, VAN, SXT, RIF	1/6/06	21 (S)	2 (S)	21 (S)	2 (S)	IPM, VAN	Favorable

[a] Results for carbapenem-resistant isolates are shown in boldface. Screening for MBL was performed using disks containing 1 μmol EDTA 15 mm from disks containing imipenem, meropenem, and ceftazidime.

[b] —, no underlying conditions; AH, arterial hypertension; SK, smoking; AMI, acute myocardial infarction; SCZ, schizophrenia; COL, colectomy; DM, diabetes mellitus; CRF, chronic renal failure; COPD, chronic obstructive pulmonary disease; DV, diverticulosis; HF, heart failure; VL, vesicular litiasis.

[c] ICU, intensive care unit; GW, general ward; GW/S, general ward/surgery.

[d] ABS, abdominal sepsis; EVS, endovascular sepsis; VAP, ventilator-associated pneumonia; SSTI, skin and soft tissue infection; OS, osteomyelitis, CAI, catheter-associated infection; PSP, postsurgical peritonitis.

[e] AF, abdominal fluid; BL, blood; CAT, catheter; BAL, bronchoalveolar lavage; BO, bone; ST, soft tissue.

[f] SAM, ampicillin-sulbactam; TZP, piperacillin-tazobactam; CIP, ciprofloxacin; IPM, imipenem; VAN, vancomycin; SXT, trimethoprim-sulfamethoxazole; AMK, amikacin; GEN, gentamicin; CRO, ceftriaxone; CAZ, ceftazidime; CLI, clindamycin; CFZ, cefazolin; ERY, erythromycin; RIF, rifampin; CST, colistin.

[g] DD, disk diffusion; R, resistant; S, susceptibility; I, intermediate.

an iodometry-based overlay system (8); no differences could be seen between both groups of isolates, and no suggestion for any other acquired β-lactamase or overexpression of the chromosomal AmpC could be inferred. No significant difference could be found between both groups of isolates concerning spectrophotometric enzymatic activity (EA), expressed as "total" β-lactamase activity (using cephalothin [CEF] as a reporter), and carbapenemase activity (using imipenem as reporter). EA for cephalothin (EA_{CEF}) (U/mg protein) for IPM-resistant isolates and IPM-susceptible isolates were $(1.7 \pm 0.3) \times 10^{-7}$ and $(2.2 \pm 0.7) \times 10^{-7}$, respectively. EA for IPM (EA_{IPM}) (U/mg protein) were $(2.7 \pm 0.6) \times 10^{-07}$ and $(4.2 \pm 2.0) \times 10^{-7}$ for resistant and susceptible isolates, respectively. Hydrolysis of imipenem relative to cephalothin (AE_{IPM}/AE_{CEF}) was 1.5 versus 1.8 in the resistant and susceptible groups, respectively, suggesting there is no difference in the level of expression of carbapenemases between IPM-resistant and IPM-susceptible isolates.

Resistant isolates also lacked a 46-kDa band in outer membrane protein (OMP) profiles (assumed as OprD) (1, 2, 7).

In vitro analysis for the presence of a high-level resistance subpopulation in two carbapenem-susceptible (IMP-13 producing) isolates (isolates 3 and 7) showed that both MEM and IPM at 16 μg/ml could select fully resistant subpopulations at frequencies ranging from 2×10^{-8} to 1.6×10^{-7} from overnight cultures when cultured on antibiotic-containing agar plates. Independently of the selecting antibiotic, they displayed resistance to both carbapenems. Not a single colony could be obtained by single-step selection from reference strains (ATCC 9027) or clinical isolates without IMP-13 at this concentration.

Mortality among patients with IMP-13-producing (IPM-susceptible) *P. aeruginosa* infections treated with IPM alone or in combination was 5/8. As this analysis is retrospective, whether subpopulations of fully resistant microorganisms that resulted in treatment failures may have also emerged in those patients (as actually occurs *in vitro*) cannot be ruled out.

We suggest MBL producers with MICs in the susceptible range should be clearly reported (categorized) differently, indicating the discordance between susceptibility and the presence of a resistance marker, even if their resistance levels do not anticipate therapeutic failure. This categorization would help to redefine the current knowledge on the epidemiological analysis comparing the evolution of patients infected by microorganisms classified as susceptible to carbapenems, but which prove to be IMP producers. Furthermore, this approach may contribute by providing data for discussing if, eventually, the presence of this associated resistance mechanism should modify current CLSI breakpoints (3).

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